REVIEW

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Process or perish: quality control in mRNA biogenesis

Milo B Fasken & Anita H Corbett

Production of mature mRNAs that encode functional proteins consists of a highly complex pathway of synthesis, processing and export. Along this pathway, the mRNA transcript is scrutinized by quality control machinery at numerous steps. Such extensive RNA surveillance ensures that only correctly processed mature mRNAs are translated and precludes production of aberrant transcripts that could encode mutant or possibly deleterious proteins.

A critical step in gene expression is the mRNA-mediated transfer of genetic information from the nuclear genome to the cytoplasm, where it can be translated into proteins. The production of a mature mRNA is a multistep process involving transcription, numerous processing events and association of various RNA-binding and processing proteins to form a mature mRNA ribonucleoprotein (mRNP) complex[1]. The mature mRNP must then be exported through the nuclear pore complex (NPC) to the cytoplasm for translation.

Advances in our understanding of mRNA biogenesis have challenged the notion that mutations within genomic coding regions are the sole cause of mutant phenotypes. It is now clear that changes or errors anywhere within the transcribed region of the genome can have profound consequences for mRNA metabolism[2–4]. Mutations in the transcript can target it for destruction and thus dictate whether the encoded protein is ever produced. Notably, errors in mRNA transcripts can occur even in the absence of any mutation in the genomic DNA sequence via error-prone transcription, incorrect processing or improper assembly of the mRNP complex[5,6]. As an example, Alzheimer disease has been linked to dinucleotide deletions in transcripts that arise by molecular misreading of correct DNA sequences and consequently code for mutant +1 proteins[7,8].

Given that errors do arise in mRNA transcripts, it is tempting to speculate that cells could be filled with defective mRNAs, which have the potential to be translated into aberrant proteins. However, cells avoid the accumulation of these defective mRNAs by using a cadre of mRNA quality control mechanisms[9–12] that rapidly recognize and eliminate the vast majority of mRNAs containing errors. These mRNA surveillance mechanisms serve a critical role. In particular, evidence now links changes in error-induced mRNA metabolism with many human diseases[2–4] and suggests they are the primary basis for the disease phenotypes. Specific examples include β-thalassemia[13] and Marfan syndrome[14] where generation of aberrant mRNAs and their targeted destruction has been linked to the diseases. In addition, some cancers and inherited genetic disorders are associated with DNA mutations or RNA editing errors that give rise to corresponding mRNAs that can trigger the mRNA quality control machinery and result in cells lacking the encoded protein altogether[2].

Research from several laboratories has provided insights into the cellular mRNA quality control machinery (**Fig. 1**). The nuclear exosome is a complex of ribonucleases that plays a critical role in monitoring the integrity of mRNA transcripts in the nucleus[9,11]. In addition, the nuclear exosome also degrades normal mRNA transcripts that accumulate within the nucleus through a process termed decay of RNA in the nucleus or DRN[15]. Two translation-dependent processes complement the function of the nuclear exosome. The nonsense-mediated decay (NMD) machinery recognizes mRNAs that contain premature termination codons (PTCs) and other imperfections[12,16] whereas the nonstop decay machinery recognizes transcripts that lack a stop codon altogether[17,18]. Interestingly, recent work has also uncovered a quality control step located at the nuclear pore[19–22], the site of transit between the cytoplasm and the nucleus[23]. This review briefly describes the various mRNA quality control mechanisms that probably function from the moment transcript is synthesized to the time the mature processed mRNA is translated at the ribosome.

mRNA life cycle

In eukaryotes, DNA is transcribed to heterogeneous nuclear RNA (hnRNA) in the nucleus by RNA polymerase II[24]. During and after transcription[25], hnRNA is extensively processed by the addition of a 5′-methyl guanosine cap, splicing of introns, and cleavage and polyadenylation of the 3′ end[1,26]. In the course of these processing events, a variety of mRNA-binding and processing factors interact with the maturing transcript[27–29]. Some of these proteins remain associated with the mRNA to form a mature mRNP that can then be released from the site of transcription and exported through the NPC into the cytoplasm[30,31]. Once in the cytoplasm, the mature mRNP complexes and the associated mRNA have several potential fates. They can associate with ribosomes for productive translation of the mRNA into proteins, they can be sequestered in specific locations such as stress granules for future use[32,33] or they can be destroyed via degradation[34]. Additionally, in some cell types, such as neurons, the mRNPs can also be targeted to specific

Department of Biochemistry, Emory University School of Medicine, 1510 Clifton Road, NE, Atlanta, Georgia 30322, USA. Correspondence should be addressed to A.H.C. (acorbe2@emory.edu).

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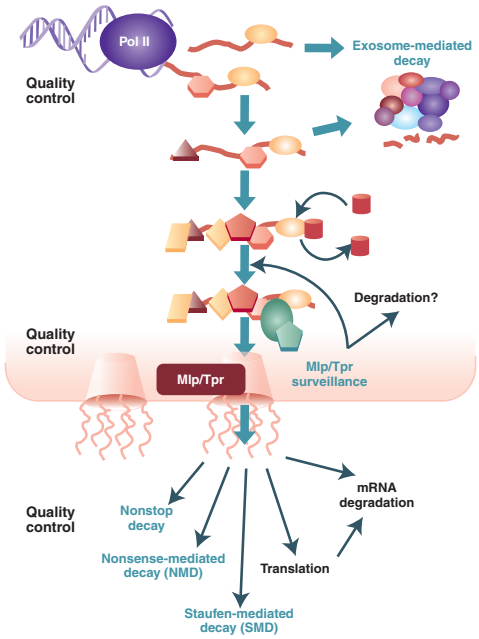

Figure 1 Schematic of mRNA biogenesis and quality control. The schematic summarizes the various known steps in mRNA biogenesis at which transcripts can be subject to quality control. Various mRNA processing factors are shown associated with the transcript. Export factors, which bind prior to export, are indicated by the green shapes. See text for details. Each quality control step in the model is indicated by a green arrow.

subcytoplasmic domains where translation may occur either spontaneously or in response to a specific signal[35]. The majority of mRNAs must also face the competing activities of translation and degradation. Ultimately, all mRNA transcripts are degraded and the mRNA pool must be replenished by newly processed and exported transcripts.

Nuclear surveillance mechanisms

One of the major RNA processing and degradation machines in the cell is the exosome complex. The exosome consists of ten proteins (**Table 1**), at least nine of which may function as 3′→5′ riboexonucleases[36]. The exosome is absolutely required for processing of some species of ribosomal RNA (rRNA), small nucleolar RNA (snoRNA) and small nuclear RNA (snRNA)[36,37]. In addition, the exosome degrades mRNAs in both the nucleus[9,11,36] and the cytoplasm[38]. It was only recently recognized that the nuclear exosome plays key roles in transcription-coupled surveillance of mRNA[9,11] and possibly the subsequent release of the transcripts from the site of transcription[39,40]. In addition, exosome function is required for decay of normal RNAs that are retained in the nucleus[15].

The first evidence for the quality control function of the nuclear exosome came from the observation in yeast that deletion of the essential *RRP6* (ribosomal RNA processing 6) gene[41], which encodes an additional 3′→5′ riboexonuclease exosome subunit localized exclusively to the nucleus and the nucleolus (**Table 1**)[36,37,42], could suppress a poly(A) polymerase mutant, *pap1-1* (ref. 43). This study revealed that Rrp6 was required for the degradation of transcripts with abnormal poly(A) tails. In fact, the nuclear exosome has now been implicated in the degradation of several types of aberrant transcripts, including unspliced pre-mRNAs[44], transcripts with a variety of abnormal 3′ ends and poly(A) tails[43,45], and even transcripts that do not acquire the correct complement of mRNA-binding proteins during transcription[46]. Additionally, exosome function, or at least Rrp6, may be required for both accumulation of aberrant transcripts in nuclear foci at or near the site of transcription and the subsequent degradation of those transcripts[39,40,45]. Thus, the nuclear exosome probably helps to assure that aberrant transcripts are detected early in their genesis and destroyed before they leave the site of transcription and waste further energy on their maturation.

Recent studies have also revealed that yeast Rrp6 is required for the degradation of apparently normal RNAs that are retained in the nucleus through a mechanism referred to as the DRN system[15]. In addition to Rrp6, the yeast DRN system depends on the Rat1 exonuclease and Cbc1, a component of the cap-binding complex, CBC. Currently it is not known whether the DRN pathway can also target aberrant transcripts and thus might overlap with exosome-mediated surveillance of abnormal transcripts. The role of Cbc1 in exosome-mediated degradation of aberrant transcripts has not yet been investigated so this feature could distinguish the two decay mechanisms. One possibility is that the DRN system may target nascent RNAs that remain within the nucleus independent of whether they are properly processed. Rapid processing and export could thus explain in part how normal nascent mRNAs avoid destruction by the exosome. It is certainly conceivable that normal as well as aberrant transcripts that spend an extended time in the nucleus could become subject to degradation by the nuclear exosome.

Table 1 Exosome components

Name		3′→5′ riboexonuclease activity[36]	Localization[42]	Essential in yeast
Yeast	Human			
Rrp4p	EXOSC22	Experimental	Cytoplasm/nucleus/nucleolus	Yes
Ski6p (Rrp41p)	EXOSC4	Experimental	Nucleus/nucleolus	Yes
Dis3p (Rrp44p)	KIAA1008	Experimental	Cytoplasm/nucleus/nucleolus	Yes
Rrp40p	EXOSC3	Predicted	Nucleus/nucleolus	Yes
Rrp42p	EXOSC7	Predicted	Cytoplasm/nucleus/nucleolus	Yes
Rrp43p	EXOSC8	Predicted	Cytoplasm/nucleus/nucleolus	Yes
Rrp45p	PMSCL1	Predicted	Nucleus/nucleolus	Yes
Rrp46p	EXOSC5	Predicted	Cytoplasm/nucleus/nucleolus	Yes
Mtr3p	EXOSC6	Predicted	Cytoplasm/nucleus	Yes
Csl4p (Ski4)	EXOSC1	No predicted exonuclease activity	Cytoplasm/nucleus/nucleolus	Yes
Rrp6p	PMSCL2	Experimental	Nucleus/nucleolus[41]	No

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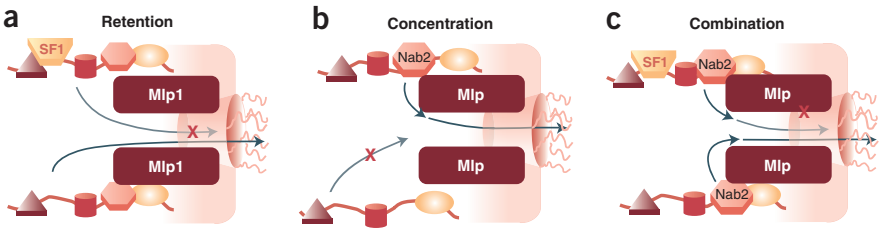

Figure 2 Models for Mlp/Tpr protein function in mRNA quality control at the nuclear pore. Various processing factors (shown associated with the transcript) bind to the maturing mRNA prior to export. Some factors remain bound during export and others are lost prior to export. See text for details. (**a**) In the retention model, transcripts are retained at the pore through interactions with Mlp1 until all processing is complete. (**b**) In the concentration model, processed mRNAs are preferentially targeted to the pore through interactions with proteins that mark them as mature. (**c**) In the combination model, discrimination would occur at the step of pore targeting and retention of incomplete or improper transcripts.

In the case of aberrant mRNAs, processing could be delayed owing to defects in the nascent transcript. Thus, a competition model for mature mRNA synthesis arises when processing events compete with exosome-mediated degradation, essentially a case of process or perish.

It is not yet understood how mRNAs undergoing maturation within the nucleus are recognized and targeted for degradation by the nuclear exosome. One possibility is that all transcripts are potential substrates for the exosome, but those that are correctly processed have a complement of associated proteins that protects them from exosome-mediated degradation. In particular, a recent study indicates that components of the nuclear exosome are colocalized with highly transcribed regions of the genome in *Drosophila melanogaster*[47]. This finding supports the notion that the exosome associates with transcripts very early in the course of transcription. In the case of DRN, it is possible that although the transcripts are presumably properly processed, their accumulation could deplete the RNA-binding factors required to protect the nascent mRNA from the exosome.

A quality control check at the nuclear pore complex

Once transcripts are synthesized and processed within the nucleus, they must be transported to the cytoplasm, where they can be translated into protein[30]. This transport is mediated by mRNA export factors, which target the transcripts to NPCs for translocation to the cytoplasm[23]. In recent years, many lines of research have revealed some of the complexities involved in the creation of an export-competent processed mRNP complex[28,29]. During the course of maturation, a transcript interacts with many proteins including processing factors, hnRNP proteins and export factors[48,49]. Some of these associations are transient: the protein interacts with the transcript, completes its assigned task and then dissociates from the complex. Other associations, however, are more extended, with the bound proteins remaining associated with the transcript throughout its export. Although many of these associated proteins are probably lost at the onset of translation, some stay bound and play a role in the translation process[50]. Our current understanding of how various factors contribute to mRNA export has been covered in several excellent reviews[28–31,51–54].

It is now appreciated that assembly of the export mRNP is intimately linked to the quality control of the transcript[28–30]. Thus, it is possible that errors within the transcript, be they changes within the coding sequence or failures in processing steps, may be reflected in the complement of specific proteins that associate with the transcript and hence the composition of the mature mRNP. In fact, the proteins that remain associated may themselves, even on an otherwise perfect transcript, become subject to scrutiny and detection of incorrect complex assembly at this level could trigger degradation. An example of this phenomenon has been reported in *Saccharomyces cerevisiae* for transcripts that do not properly load the mRNA-binding proteins Sub2 and Yra1 (ref. 46).

Although distinct steps in mRNA biogenesis may exist within the nucleus, a critical transition is clearly made when the transcript moves from the nucleus to the cytoplasm. This transition is an important irreversible step in the maturation of an mRNA transcript. For this reason, it seems likely that the movement from the nucleus to the cytoplasm may be monitored in some way. In support of this idea, several recent studies have identified proteins at the nuclear face of the NPC that assure that only mature, fully processed mRNAs are exported from the nucleus[52]. These proteins are known as myosin-like proteins (Mlp1 and Mlp2) in budding yeast[55,56] and as translocated promoter region (Tpr) protein in vertebrates[57,58].

The yeast Mlps and vertebrate Tpr are localized to the inner basket of the NPC[56,59]. These proteins are thus favorably located to make contact with an mRNP as it is targeted for export from the nucleus. The first evidence that the Mlp and Tpr proteins might play some role in mRNA export stemmed from the finding that overexpression of either Mlp1 or Tpr causes mRNA to accumulate in the nucleus[59,60]. Subsequent studies revealed a direct interaction between Mlp1 and the yeast hnRNP protein Nab2 (ref. 20). This study suggested that the nonessential Mlp proteins might monitor mRNP complexes to assure that they are suitable for export to the cytoplasm. A more recent study by Galy *et al.* has uncovered a specific role for Mlp1 in mRNA quality control by demonstrating that Mlp1 mutants allow unspliced transcripts to leak to the cytoplasm[19]. The study by Galy *et al.* also identified an RNA-dependent interaction between Mlp1 and a splice site–binding protein, SF1 or branch-point-binding protein (BBP)[61], which led to a model where mRNAs are retained in the nucleus until processing is completed[19]. This retention model (**Fig. 2a**) suggests that an mRNP complex remains bound to the Mlp1 platform and hence tethered at the inner face of the nuclear pore until splicing is completed. Only after splicing, and perhaps other processing, is complete, is the mRNP released for export to the cytoplasm. A second model that differs slightly in the details has also been proposed[20,21]. In this concentration model (**Fig. 2b**), the Mlp proteins preferentially interact with fully processed transcripts to concentrate them at the nuclear pore for export[20,21]. These interactions would be mediated by mRNA-binding proteins, such as Nab2, that could serve as markers for the completion of specific processing events.

Although these two models differ somewhat in the details, they both invoke a role for Mlps in monitoring the maturity of the mRNAs to be exported to the cytoplasm. One possibility is that Mlps survey mRNA export through a mechanism that combines these two models. In this combination scenario (**Fig. 2c**), mature or maturing messages are targeted to the Mlps through interactions with hnRNPs, such as Nab2 or export factors, that are loaded during processing. Once these transcripts associate with the Mlps, the presence of splicing factors such as SF1 could mediate retention until the splicing factors are lost, as proposed by Galy *et al*[19]. This model would suppose a two-step mechanism consisting of concentration of export-competent transcripts at the pore followed by discrimination to assess the processing status of the transcript and hence its suitability for export. In support of this combination model, Vinciguerra *et al.* have recently shown that Mlp2 binds to the mRNA

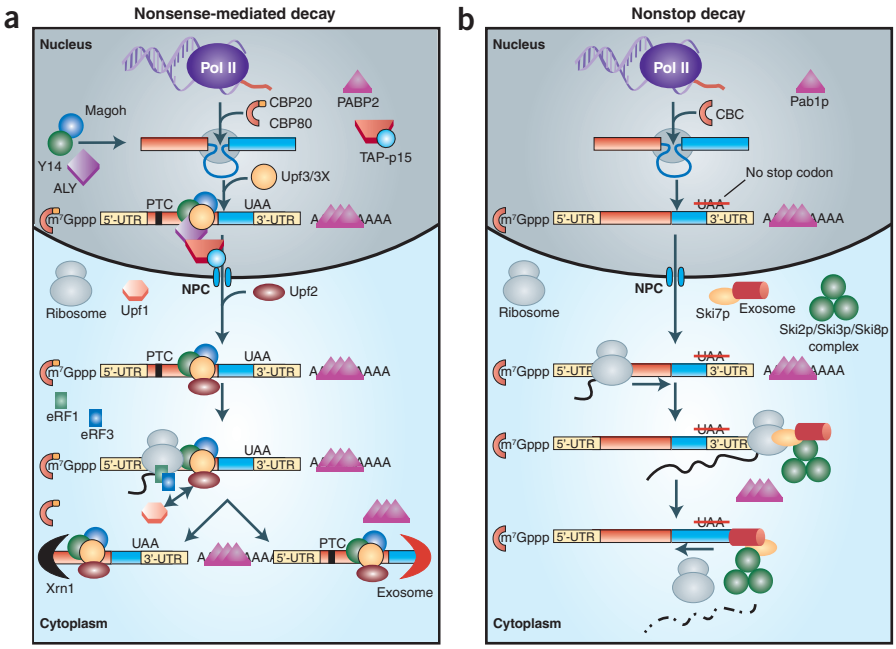

Figure 3 Models for NMD pathway in higher eukaryotes and nonstop decay pathway in yeast. (**a**) NMD. After splicing and deposition of the EJC, including Y14, Magoh and ALY, onto an mRNA, the predominantly nuclear NMD protein Upf3/3X joins the complex. After nuclear export of the mRNA, some EJC components dissociate and the cytoplasmic NMD protein Upf2 binds the complex via interactions with Upf3/3X. During a pioneer round of translation, NMD is triggered by recognition of a PTC upstream of an exon-exon junction and the EJC-associated NMD proteins Upf2, Upf3/3X and Upf1. The cytoplasmic protein Upf1 probably joins the EJC last during translation termination. The PTC-containing mRNA is then degraded from the 5′ end by decapping and Xrn1-mediated 5′→3′ exonucleolytic decay or from the 3′ end by deadenylation and exosome-mediated 3′→5′ exonucleolytic decay. In *D. melanogaster*, the mRNA can be endonucleolytically cleaved in the vicinity of the PTC and the resulting mRNA fragments degraded by 3′→5′ and 5′→3′ decay. (**b**) Nonstop decay. After splicing and export of the nonstop mRNA, the transcript is translated by the ribosome. Without a termination codon, the elongating ribosome proceeds through the poly(A) tail, displaces Pab1, and stalls at the 3′ end of the mRNA. The now empty ribosomal A site is recognized by Ski7p, which harbors a C-terminal domain similar the GTPase domains of elongation and termination factors EF1A and eRF3. Ski7p binding results in the recruitment of the exosome and the Ski2p–Ski3p–Ski8p complex, and dissociation of the ribosome. The nonstop mRNA is then degraded from the 3′ end by the exosome and the protein product is destroyed.

export factor Yra1p, but interacts more strongly with a Yra1p mutant that causes mRNA export defects[21]. These results suggest that Mlp2 recruits wild-type Yra1p to facilitate mRNP docking, but selectively retains malformed mRNP complexes containing the Yra1p mutant[21]. One challenge will be to determine how the Mlp surveillance mechanism is linked to other mRNA export factors, such as Mex67/TAP, that are clearly required for mRNA export from the nucleus[49].

It is worth noting that one difference between other quality control mechanisms described here and the Mlp-mediated step is the lack of any apparent mechanism to target aberrant transcripts for degradation. As yet, no ribonuclease activity has been associated with the function of the Mlp proteins, but there could be an Mlp-triggered or associated nuclease activity that has not yet been identified. At present, the Mlp/Tpr proteins are considered 'gatekeepers'[22] that survey the transcripts and impact their movement without any direct means to dispose of mRNAs that fail to make the grade[52].

Nonsense-mediated decay and nonstop decay

Both translation-dependent RNA surveillance mechanisms, NMD and nonstop decay, depend on some method of decoding the transcript to detect the presence of stop codons. NMD is best known for its ability to target transcripts that contain PTCs for decay[12,62,63]. In contrast, nonstop decay targets transcripts that lack a stop codon for destruction[17,18].

Nonsense-mediated decay. The NMD pathway was first identified through genetic screens in *S. cerevisiae*[64] and *Caenorhabditis elegans*[12,65,66]. The screen carried out in yeast identified the primary components, Upf1, Upf2 and Upf3, on the basis of their ability to suppress a +1 frameshift mutation in the *HIS4* gene (Up-frameshift)[64]. Genetic analysis in worms identified a larger family of genes referred to as the *smg* (suppressor with morphogenic effect on genitalia) genes[66]. Whereas SMG-2, SMG-3 and SMG-4 are homologs of Upf1, Upf2 and Upf3, respectively, SMG-1, SMG-5, SMG-6 and SMG-7 have no apparent yeast homologs (**Table 2**). Importantly, all these NMD components, except for SMG-7, have also been identified in flies. Work thus far indicates that the primary role of the additional SMG proteins is to regulate a phosphorylation cycle of Upf1 in higher eukaryotes[65,67–69]. Recent work indicates that human SMG-7 acts as an adaptor to recruit phosphorylated Upf1 and target its associated mRNA for degradation in cytoplasmic mRNA decay bodies[70,71]. However, there is no evidence that Upf1 is a phosphoprotein in yeast[65], suggesting that regulation and recruitment of Upf1 may differ in this organism.

The NMD machinery recognizes not only transcripts containing PTCs but also aberrant transcripts with incomplete splicing[72], extended 3′ untranslated regions (UTRs)[73] or upstream open reading frames in their 5′ UTRs[74,75] (**Fig. 3a**). These NMD substrates are targeted for degradation via interaction with the Upf proteins[12,62,63]. The Upf3 protein, which primarily localizes to the nucleus in mammalian cells[76,77], probably binds first to NMD targets[76]. After export to the cytoplasm, Upf2 joins these transcripts by binding to Upf3 (refs. 12,63,78). Key molecular details of the Upf2–Upf3 complex were recently revealed by the resolution of a crystal structure of the human Upf2-Upf3 interaction domains[79]. Upf1 probably associates with Upf2 and the NMD complex last to trigger destruction of the nonsense-containing transcripts[12,63,80]; however, the molecular mechanism of Upf1 function in NMD is not yet fully understood.

Unlike the exosome, the NMD machinery does not have inherent exonuclease activity. Instead, it marks aberrant transcripts for degradation, which can proceed through several pathways[81,82]. In one NMD-dependent pathway, the transcript is decapped and degraded by the 5′→3′ Xrn1 exonuclease[73,83,84]. In a second pathway, the transcript is degraded 3′→5′ through an exosome-dependent mechanism[82,85]. Finally, in a third and recently identified NMD-triggered decay pathway in *D. melanogaster*, the transcript is endonucleolytically cleaved in the vicinity of the nonsense codon followed by degradation of the 5′ fragment by the exosome and the 3′ fragment by Xrn1 (ref. 86). The identification of this latter NMD pathway in *D. melanogaster* raises the

possibility that although NMD is evolutionarily conserved, the mechanistic details could differ between organisms.

Like the exosome, the NMD machinery functions in several cellular processes. The Upf proteins have been implicated in translation termination partly because they directly interact with the translation elongation factors eRF1 and eRF3 (ref. 12). Such a link between NMD and translation is not unexpected as translation is required to identify nonsense codons within the coding sequence of the mRNA[12]. Indeed, there is considerable evidence that NMD depends on ongoing translation[12,16]. In addition to a link with translation termination, the three *C. elegans* SMG (Upf) proteins, SMG-2 (Upf1), SMG-5 and SMG-6, have also been implicated in RNA interference[87].

Much effort in recent years has focused on understanding how the NMD machinery recognizes transcripts as aberrant, particularly those that contain a PTC, and how these transcripts are then marked and subsequently targeted for degradation. Efforts to define *cis*-acting sequences within the transcript and *trans*-acting factors that trigger NMD have revealed rather different results in yeast and mammals. However, in all organisms, a combination of two *cis* elements is required to trigger NMD[65]. The first *cis* element is an abnormality in the mRNA, such as a PTC, which is required in all species and the second is an additional RNA element—an exon-exon junction in mammals or downstream sequence element (DSE) in yeast. Certainly, one major difference in NMD among species is that in mammals, splicing, and hence the presence of an exon-exon junction, is required for NMD[65]. Thus, in general, nonspliced messages are not subject to NMD in mammals[88]. In contrast, non-intron-containing messages are subject to NMD in *S. cerevisiae*, *C. elegans* and *D. melanogaster*[65].

Studies on NMD in yeast and mammals have begun to elucidate how PTCs can be recognized and have revealed some key mechanistic differences between these species[65,89]. In yeast, there is evidence that, in conjunction with a PTC, an RNA sequence element termed the DSE within the normal coding region is recognized by a surveillance complex that recruits the Upf proteins[90]. Only one component of this proposed pathway has been identified, the essential hnRNP, Hrp1/Nab4 (ref. 90). Hrp1 functions in the cleavage of the 3′ ends of transcripts within the nucleus and shuttles, presumably together with the mRNA, to the cytoplasm[91]. It should be noted that the evidence in support of this model comes from a detailed analysis of a single DSE[90]. This DSE is sufficient for NMD targeting of a mini-gene substrate but is not absolutely necessary for NMD-targeted degradation of a full-length phosphoglycerate kinase (PGK1) transcript. Another model proposed for the NMD mechanism in yeast is that an abnormal 3′ UTR, such as an elongated 3′ UTR, together with the PTC triggers NMD[6,65]. Clearly, one possibility is that a combination of these RNA elements triggers NMD. Abnormally long 3′ UTRs could easily contain as yet undefined DSEs. Further research is required to understand precisely how the Upf proteins are recruited to aberrant transcripts in yeast.

In contrast to yeast, many proteins that mark mammalian transcripts for NMD have been identified. These proteins form a complex known as the exon junction complex (EJC)[92]. As implied by its name, it is composed of proteins that associate with the transcript in the course of splicing[27,69,93]. Thus, in mammals the exon-exon junction is the *cis*-acting sequence that acts in conjunction with the PTC to trigger NMD[12,27,62]. Experiments indicate that when a termination codon is located >50–55 nucleotides upstream of an exon-exon junction, that termination codon is recognized as a PTC and the transcript is targeted for degradation[27,94]. A current model suggests that recognition of a PTC relative to the position of the proximal EJC occurs during ribosome scanning of the transcript in a 'pioneer round' of translation[12,95]. After this pioneer round, either the transcript is targeted for degradation by the NMD machinery if a PTC is identified, or, if no defects are detected, it is shifted into productive protein translation mode (**Fig. 3a**).

Although NMD is restricted to the cytoplasm in yeast[15,81,96], there is evidence in higher eukaryotes for nucleus-associated NMD[12,62]. It is, however, unclear from the biochemical fractionation experiments that were used to detect this nuclear NMD activity whether the NMD functions in the nucleoplasm or whether it targets messages undergoing nuclear export[65]. If NMD were to take place in the nucleus as has been speculated, this would invoke a requirement for nuclear translation, a subject that is controversial and yet to be resolved[97,98]. With respect to this question, it is notable that several NMD proteins can shuttle between the cytoplasm and the nucleus (**Table 2**). This finding suggests that certain NMD proteins, such as Upf3 (ref. 76), could function or possibly load onto transcripts within the nucleus and lends weight to the possibility that nuclear NMD could occur.

Interestingly, a novel mRNA decay pathway that is distinct from NMD, termed Staufen 1–mediated mRNA decay (SMD), has recently been identified in mammals[80]. The SMD mechanism involves the NMD factor Upf1, the RNA-binding protein Staufen (Stau) 1 and a PTC[80]. In contrast to NMD, SMD does not require splicing or an EJC[80]. During SMD, mammalian Stau1 binds the 3′ UTRs of a subset of mRNAs that

Table 2 NMD components

Name				Activity/function/domains	Localization[42]	Essential in yeast
Yeast	Mammal	Worm	Fly			
Upf1p (Nam7p)	Upf1	SMG-2	Upf1-PA	RNA-dependent ATPase/3′→5′ helicase	Cytoplasm/P-bodies[104]/nuclear shuttling[105]	No
Upf2p (Nmd2p)	Upf2	SMG-3	Upf2-PA	Nucleic acid binding (predicted)	Cytoplasm/nuclear[106]	No
Upf3p	Upf3 and Upf3X[a]	SMG-4	Upf3-PB and Upf3-PC	mRNA binding	Nuclear/nuclear shuttling/cytoplasm[107]	No
NA	Smg1	SMG-1	Smg1-PA	Phosphatidyl kinase-related kinase	Cytoplasm[108]	NA
NA	Smg5	SMG-5	Smg5-PA	Phosphoprotein phosphatase/PP2A complex/ TPR/PIN domains	Cytoplasm/nuclear shuttling[69]	NA
NA	Smg6	SMG-6	Smg6-PA	TPR/PIN domains	Cytoplasm/nuclear shuttling[68]	NA
NA	Smg7	SMG-7	NA	Phosphoprotein phosphatase/PP2A complex and Upf1 targeting to mRNA decay bodies/ TPR domain	Cytoplasm/nuclear shuttling[69]	NA

All information cited was obtained from the Incyte database (http://www.proteome.incyte.com) and the FlyBase database (http://flybase.bio.indiana.edu) unless otherwise noted.
NA, not applicable (no obvious homolog is found in that species); TPR, tetratricopeptide repeat domain; PIN, PilT N terminus domain.
[a]Also known as Upf3A and Upf3B.

includes ADP-ribosylation factor (Arf) 1 and recruits Upf1 to target these transcripts for degradation[80]. The discovery of SMD raises the intriguing possibility that NMD factors like Upf1 may operate in diverse mRNA decay pathways.

Nonstop decay. In contrast to NMD, the nonstop decay machinery recognizes transcripts that lack a termination codon and thus could encode abnormally extended proteins[17,18,99,100]. Although nonstop decay, like NMD, relies on translation, the mechanism is distinct (**Fig. 3b**). Degradation of nonstop mRNA transcripts requires the cytoplasmic exosome, the associated Ski complex, composed of Ski2, Ski3 and Ski8, and the adaptor protein Ski7 (refs. 99,100). This pathway is independent of the Upf1 protein that is required for both NMD[99] and SMD[80]. Interestingly, this pathway is also independent of the decapping and deadenylation factors that are typically required to initiate mRNA decay[99]. Thus, it seems that the form of the exosome that functions in nonstop-triggered decay may be able to degrade the poly(A) tail. This feature makes the nonstop decay mechanism rather distinct as poly(A) tail trimming is a prerequisite for most mRNA decay mechanisms[101]. One mechanistic model for nonstop decay suggests that the C terminus of the Ski7 protein, which resembles the GTPase domain of the EF1A and eRF3 translation factors[100], interacts with the empty ribosomal acceptor site at the end of the transcript to release the ribosome and recruit the cytoplasmic exosome.

Summary and future challenges

As briefly described here, the cell has evolved multiple surveillance mechanisms to assure that only perfect mRNAs are ultimately translated into proteins. This multitude of overlapping quality control pathways guarantees that the cell has mechanisms in place to pinpoint the many types of errors that could arise in the transcript as it is polymerized, processed, exported and translated. In addition, some of the surveillance machines have overlapping specificity that enables them to catch and destroy errant transcripts in the cytoplasm that escaped quality control in the nucleus. All these pathways help to ensure the fidelity of gene expression.

Although this review focuses on quality control in mRNA biogenesis, several recent studies suggest that similar quality control machinery could exist to monitor the integrity of other classes of RNAs. In *S. cerevisiae* small pools of both tRNA[102] and rRNA[103] are subject to polyadenylation and this polyadenylation is correlated with a decreased half-life of these RNAs. These observations suggest that polyadenylation of RNAs other than mRNA could mark those RNAs for degradation by the exosome. In fact, the adenylated tRNA species is hypomethylated and thus improperly processed[102]. It is intriguing to consider the possibility that all RNA transcripts may be subject to the extensive surveillance mechanisms and use some of the same quality control components that have thus far only been described for mRNA.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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